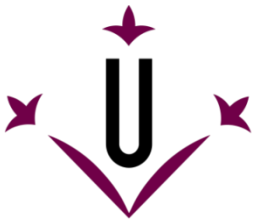

Rainfall homogenizes while fruiting increases diversity of spore deposition in Mediterranean conditions

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Abstract

There is a lack of knowledge regarding the main factors modulating fungal spore deposition in forest ecosystems. We have described the local spatio-temporal dynamics of fungal spore deposition along a single fruiting season and its relation with fruiting body emergence and rainfall events. Passive spore traps were weekly sampled during autumn and analysed by metabarcoding of the ITS2 region in combination with qPCR. There were larger compositional changes of deposited spores across sampling weeks than amongst sampling plots. Spore diversity and abundance correlated with mushroom emergence and weekly rainfall. Spore compositional changes were related to rainfall, with lower spatial compositional heterogeneity across plots during weeks with higher rainfall. Soil saprotrophs, and amongst them, puffball species, showed the strongest positive correlation with rainfall across fungal guilds. We saw high fine-scale temporal changes of deposited spores, and both mushroom emergence and rainfall may be important factors driving airborne spore deposition.

Keywords: *Fungal diversity, Atmospheric diversity, qPCR, DNA barcoding, spore traps, dispersion.*

1. Introduction

Understanding soil fungal community assemblages is essential for predicting soil nutrient dynamics and plant-soil feedbacks. A general framework suggests that both competition between fungi and colonization processes structure fungal assemblages, sustaining hyper-diverse communities (Smith et al., 2018). Competition processes in fungi are largely determined by host, substrate and habitat availability, but fungal communities are also modulated by climate (Botnen et al., 2019; Castaño et al., 2018), soil parameters (Rincón et al., 2015) or disturbances such as tree harvesting (Kohout et al., 2018; Tomao et al., 2018) or fire (Clemmensen et al., 2013). However, the high spatial stochasticity found in soil fungal communities suggests that dispersal processes may be extremely important in determining community assemblages (Bahram et al., 2016). Both stochastic and predictable processes driving spore dispersal may affect fungal colonization outcomes (Peay et al., 2012; Peay and Bruns, 2014), which may in turn cause a cascade effect on the belowground community build-up (Kennedy et al., 2009). Spore deposition may potentially be influenced by processes such as atmospheric conditions (e.g. wind, precipitation; Oliveira et al., 2009; Despres et al., 2012; Dam, 2013) or by inter-specific differences in spore dispersal abilities (Peay et al., 2012; Peay and Bruns, 2014). For instance, dispersal limitation observed in several fungal species (Galante et al., 2011) suggests that colonization in a given landscape may be highly determined by the surrounding local fruit body communities, followed by niche competition between successfully deposited fungal spores. Considering the ecological importance of all these processes, further research on spore dispersal and spore deposition needs to be carried out.

Fruit bodies from basidiomycetes produce billions of spores (Dahlberg and Stenlid, 1994; Kadowaki et al., 2010), which will deposit by gravity near the sporocarps (Galante et al.,

2011; Peay et al., 2012; Peay and Bruns, 2014). A small proportion of these spores may be dispersed over long distances when reaching the turbulent layers of the atmosphere (Dressaire et al., 2016). In the turbulent layer, spores from many sources located over relatively large areas are mixed (Lacey and West, 2006; Nicolaisen et al., 2017). In contrast to short distance spore deposition, it is likely that long distance spore dispersal is determined by environmental factors such as wind or UV light (Burch and Levetin, 2002; Kivlin et al., 2014; Nicolaisen et al., 2017). Among these environmental factors, rainfall may also be a relevant process that potentially affects spore dispersal and deposition of airborne spores (Oliveira et al., 2009, Pakpour et al., 2015). Rainfall effects on spore deposition may be especially relevant in drier climates such as Mediterranean, where mushroom emergence is seasonal and concentrated in autumn months when rainfall is more abundant (Martinez de Aragón et al., 2007; Alday et al., 2017). Within this context, spore deposition of some fungal species during autumn may be restricted to a few days, during rainfall, whereas for other species dry deposition (gravity) may be predominant and, therefore, deposition occurs regardless of rain. In any case, the role of rainfall affecting spore deposition (i.e. spore community and diversity) compared with dry deposition is still unknown, mostly due to technical limitations related with species-typing of spore pools.

By using filter or funnel traps it is possible to profile and describe the spore or propagule community that would be deposited on the ground (Peay and Bruns, 2014). Molecular identification and composition profiling of these spores may be then achieved using high-throughput DNA sequencing (HTS) techniques (Aguayo et al., 2018). HTS data is adequate for quantitative purposes, as shown by qPCR using specific fungal species (Oliva et al., 2017). By profiling the spores in rainfall funnel traps, we previously observed that specific spore deposition of some species was correlated with mushroom production, regardless of rainfall events (Castaño et al., 2017). This finding indicated that spores were released and

deposited as fruiting bodies were formed. However, correlation between mushroom production and deposited spores did not match for several species, pointing out the existence of other factors involved in spore deposition. At the same time, in our previous study (Castaño et al., 2017) we did not work with the whole spore pool, focusing only on selected species that produced fruit bodies at the studied plots. In addition, questions regarding how rainfall affects airborne spore deposition or the compositional changes of spore communities across plots and weeks were not targeted. However, in this study, we investigated the role of rain and mushroom emergence on spore deposition by concomitantly describing funnel trap captures and fruiting in a local scale Mediterranean forest during a single fruiting season. We hypothesized that rainfall will homogenize spore deposition across plots (lower spatial compositional heterogeneity across plots) by collecting spores from the turbulent layer. In this research, we have (i) tested whether compositional changes in deposited spores across weeks (deposited spore succession) is higher than compositional changes from spatial relatedness, both considering the whole spore composition and splitting it into functional groups. In addition, we have (ii) described how rainfall and mushroom emergence contributed to temporal changes in spore deposition at local scale (i.e. total abundance, composition and diversity).

2. Material and Methods

2.1 Study area

The study was carried out at the long-term experimental forest located in the Natural Area of Poblet (Northeast Spain, 41° 21’ 6.4728” latitude N and 1° 2’ 25.7496” longitude E). The site has a Mediterranean climate, with an average annual temperature of 11.8°C and annual rainfall of 665.5 mm., with summer droughts usually lasting for 3 months. In this study, we

used 8 randomly chosen plots (10 × 10 m) of an experimental set-up where fruit bodies have been continuously monitored every fall since 2008 (Bonet et al., 2012). This set of plots was located in a forest area covering approximately 300 ha (Fig. S1a). Forests are even-aged (60-y-old) *Pinus pinaster* trees with *Quercus ilex* as a co-dominant species. Spore trap and mushroom samples were obtained in these plots during a single autumn season. Sampling and identification of mushrooms at the study site is described in Castaño et al., (2017).

2.2 Spore trapping

Funnel traps were installed in 8 plots, 30 cm above ground level. Traps consisted of 15 cm diameter plastic funnels attached to 1-L dark jars, with a 50-µm nylon mesh fixed at the bottom of the funnel. Traps were installed 1 week after the first fruit body was observed (October 14th, 2014) and remained in the plot until December 11th, 2014. Funnel traps were rinsed with ultrapure water (MilliQ) each week to collect any spores attached to the funnel. After that, the jars were replaced with new sterile jars. Spore suspension in the jar was filtered using sterile filter papers (90-mm diameter: Whatman no. 5) within 48 h of collection. Filter papers were stored at –20ºC until further analysis.

2.3 Spore trap sample processing and DNA extraction

Filters containing spores from the funnel traps were cut in half and stored in separate 50-ml falcon tubes. A solution of 20-ml sodium dodecyl sulfate (SDS buffer) was added to each tube, and tubes were incubated at 65°C for 90 min. Tubes were then vortexed three times and filter from each tube was removed. Twenty milliliters of 2-propanol were added to the resulting solution and was left overnight at room temperature. The solution was centrifuged (750 × g. for 10 min) and the supernatant was removed. The resulting pellet was then resuspended by vortexing with 700 µl of SL2 lysis buffer (NucleoSpin® NSP soil DNA extraction kit, Macherey-Nagel, Duren, Germany) and transferred to a 2-ml tube. After the

addition of SX Enhancer (NucleoSpin® NSP soil DNA extraction kit), the spore solution was homogenized in a FastPrep®-24 system (MP Biomedicals) at 5,000 × r.p.m for 30 s (twice) and total DNA was extracted following the instructions provided by the manufacturer.

2.4 qPCR using universal fungal primers

For quantification of the fungal spores, we used the universal fungal primers ITS1F (Gardes and Bruns, 1993) and 5.8S (Vilgalys and Hester, 1990), designed to amplify the ITS1 region located in the rRNA encoding DNA. Quantitative PCR reactions were performed in triplicate including negative controls using 96-well transparent plates on an iCycler iQ thermal cycle (BioRad). Reactions contained 12.5 µL of SsoAdvanced Universal SYBR Green Supermix, 2.5 µL of bovine serum albumin (BSA: 5 ng/ µl), 0.25 µl of a 10 µM mixture of each primer, 5 µl of template (diluted extracted DNA 1:10) and sterile water to a final volume of 20 µl. Reaction conditions included 95ºC for 2 min, followed by 40 cycles of 95ºC for 15 s, annealing temperature for 30 s at 53ºC, and 72ºC for 30 s. Standard curves were prepared using a solution obtained from known number of spores of three fungal species (*Trichoderma* sp. (sticky spores), *Cryphonectria parasitica* (rainsplash spores) and *Cantharellus cibarius* (airborne spores), which were extracted using the same protocol as filters. C_T values were converted to the number of fungal spores in each reaction using serial DNA dilutions of known amounts of spores, starting with 1×10^6 spores and ending with 100 spores. Based on these standard curves, results are expressed as number of spores × trap sample $^{-1}$. For all the reactions there was a linear relationship between the logarithm of the spore number and the threshold cycle across the standard concentration range ($R^2 > 0.98$) as well as efficiency values between 95%-105%.

2.5 Spore trap sample sequencing using Illumina MiSeq

Each spore trap sample was PCR-amplified using the fungal universal primers fITS7 and ITS4 (Ihrmark et al., 2012) which amplify the ITS2 region of the rDNA. Both primers were tagged with 8-bp sequences, differing in at least three positions. The number of PCR cycles was optimised for each sample, and most of the samples amplified well at 24–26 cycles. PCR amplifications of samples and both negative controls from DNA extraction and PCR were conducted in a 2720 Thermal Cycler (Life Technologies) in 50 µl. The final concentrations in the PCR reaction mixture were; 25 ng of template, 200 µM of each nucleotide, 2.75 mM $MgCl_2$, 200 nM of each primer, 0.025 U μl^{-1} polymerase (DreamTaq Green, Thermo Scientific, Wathman, MA) in 1× buffer PCR. The cycling conditions for PCR were: 5 min at 95°C, followed by 24–30 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C, and a final extension step at 72°C for 7 min before storage at 4°C. DNA from each sample was amplified in triplicate and amplicons were purified using an AMPure kit (Beckman Coulter Inc. Brea, CA) and quantified using a Qubit fluorometer (Life Technologies, Carlsbad, CA). Equal amounts of amplified DNA from each sample were pooled before sequencing. The final equimolar mix was finally purified using an EZNA Cycle Pure kit (Omega Bio-Tek, USA). Quality control of purified amplicons was carried out using a BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA) 7500 DNA chip. Libraries were prepared from ~10 ng of fragmented DNA using the ThruPLEX-FD Prep kit. The samples were sequenced using the Illumina MiSeq platform, with 300-bp paired-end read lengths, generating 13.4 million sequences.

2.6 Quality control and bioinformatic analysis

Quality control, filtering, and clustering were assessed using the SCATA pipeline (scata.mykopat.slu.se). Sequences were filtered to remove DNA sequences with a minimum

base quality score of <10 at any position, an average quality score of <20, and a minimum sequence length of 200 bp, using the amplicon quality option. Sequences were also screened for primers (using 0.9 as a minimum proportional primer match for both primers) and sample tags. The 'usearch' was used as a search engine, considering a minimum match length of 85%. Homopolymers were collapsed to 3 bp before cluster analysis. Pairwise alignments were conducted using a mismatch penalty assigned of 1, gap open penalty of 0, and a gap extension penalty of 1. Sequences were clustered in Operational Taxonomic Units (OTUs) using single linkage clustering, defining 1.5% as a threshold distance with the closest neighbor. Finally, all sample tags were identified, and tag jumps were removed from the database. The raw sequence reads have been deposited in the NCBI Sequence Read Archive (SRA) under accession number **PRJNA352156**.

2.7 Identification of the fungal clusters

We taxonomically identified the most abundant 2,000 OTUs using the massBLASTer option in the PlutoF database (https://plutof.ut.ee/) (Abarenkov et al., 2010). The most abundant sequence from each cluster was selected for taxonomic identification. Taxonomic identities were assigned based on >98.5% similarity with database references. Functional identification of identified taxons at species or genera level was done by using FUNGuild (Nguyen et al., 2016). All filtered DNA sequences, environmental data and fungal identifications are available at Mendeley Data (DOI: **10.17632/42hhdp53zb.1**).

2.8 Climate data

We obtained daily weather variables of precipitation from 2014 for each of the 8 plots, following DAYMET methodology (Thornton et al., 2000), as implemented in the R package 'meteoland' (De Cáceres et al., 2017). In this package, daily precipitation and temperature was estimated for each plot by averaging the values of several local meteorological stations

recorded during the sampling period, weighting factors that depended on the geographic proximity to the target plot and correcting for differences in elevation between the station and the target plots. Low weekly precipitation values (<20 mm.) were recorded during the mushroom productive season, with the exception of the week between from 28th November to 4th December, that registered an average of 162 mm in less than 48 h. This extreme precipitation episode was caused by a cut-off low, associated with S-SE winds carrying also significant amounts of Saharan dust (a sequence of the geopotential maps from 28th November – 1st December can be seen in Fig. S2A, B, C, D). As a result of this rainfall event, the spore traps overflowed and some spores were probably lost.

2.9 Data analysis

Statistical analyses were implemented in the R software environment (version 2.15.3; R Development Core Team, 2013) using the "vegan" package for multivariate analysis (Oksanen et al, 2015) and "nlme" package for linear mixed models (Linear Mixed Effects models (LME): Pinheiro et al., 2016). "iNEXT" package (Hsieh et al., 2016) was used for diversity analysis and interpolation of fungal diversity data. Ordination of community data (Detrended Correspondence Analysis: DCA) was also carried out using CANOCO version 5.0 (Biometris Plant Research International, Wageningen, The Netherlands).

2.9.1 Deposited spore compositional changes across weeks

We used variation partitioning analyses to identify whether temporal (weeks identity) or spatial (plots identity) effects were significantly influencing deposited spore composition. Here, we used the "varpart" function on compositional data, down weighting the effect of rare species with Hellinger transformation (Legendre and Gallagher, 2001). We also studied the effect of geographical distance of plots (spatial autocorrelation) on the spore composition. As

a geographical distance index, we first calculated the principal coordinates of neighbor matrices (PCNM) spatial eigenvectors, based on UTM coordinates of the sampled plots, using Euclidean distances. We used forward selection of explanatory variables to select for significant eigenvectors, using Bonferroni correction of *P*-values. The scores of the significant spatial eigenvectors (named PCOs) for each plot were used as explanatory variables in the variation partitioning analyses, together with the plot and weeks identity. First, a general variation partitioning analyses was performed, but afterwards separate analyses were done over the most abundant taxonomic fungal groups (i.e. ectomycorrhizal, wood saprotrophs, plant pathogens, other saprotrophs, lichenized and soil saprotrophs). Geographical distance (PCOs), weeks and plots identity were defined as explanatory variables and significance was obtained by redundancy analysis (RDA) with 999 permutations, and stratifying by Week (Strata= Week). The graphical representation of the deposited spore similarity between weeks was obtained using a Detrended Correspondence Analysis (DCA) with 'week' fitted over the ordination. Here, one DCA for each functional group was done to facilitate the interpretation of outcomes. Also, spore temporal patterns of most abundant species commonly found in the fruit body community were assessed using General Additive Models (GAM) and visualized as response curves (16 species only). In these analyses, we included the 12 most abundant functional guilds.

2.9.2 Fruiting body and spore diversity calculations

Hill's series of diversity indices was used to identify diversity patterns of spores across weeks (Hill, 1973). Hill's diversity consists of three numbers: N0 is species richness; N1 is the exponential of Shannon's diversity index; and N2 is the inverse of Simpson's diversity index. N0, N1 and N2 were calculated from the asymptotic estimates implemented in "iNEXT" and values were derived in all samples considering the minimum number of reads observed across

all data set (Number of reads=6,625). For the fruit body community, only richness was included at the analyses.

Finally, since rarefaction may result in a potential loss of information or incorrect interpretation of results (McMurdie and Holmes, 2014), we also performed spore diversity analyses on non-rarefied samples. As an alternative to take in account the uneven read distribution, we used the square root transformation of read counts as an explaining variable when testing for the Hill's numbers (Bálint et al., 2015), and using the same as previously models. Similar results were obtained with or without rarefaction.

2.9.3 Rainfall and fruiting body effects on spore composition, abundance and diversity

Rainfall effects on weekly compositional changes of deposited spores were studied by redundancy analysis (RDA) with 200 permutations. Here, Hellinger transformed community data was considered as a response variable, whereas log-transformed precipitation data was considered as an explanatory variable. To study whether precipitation washed-out airborne spores, we calculated the compositional heterogeneity of spore communities across weeks, which was determined by calculating the area of the standard deviational ellipse resulting from weekly composition dispersion, and adjusted with the weekly precipitation using linear models. Rainfall and mushroom emergence effects on deposited spore diversity were tested with a temporal perspective using LME. LME models considered the interaction "fruit body diversity × rainfall" as a fixed variable and the plots and week identities as a random factors. LME models were also used to study relationships in a temporal scale between the relative abundance of each functional guild and the weekly precipitation, by defining precipitation values as fixed factor, plots and week identities as random factors. Relationships between rainfall and (i) spore abundance as measured by qPCR, and (ii) relative abundance of guilds

or specific fungal groups (e.g. puffballs) were also studied by LME, following the previous scheme.

Since increase of fungal spore diversity may be related to increasing number of spores washed-out by rainfall, relative proportions of each fungal species were normalized by the total number of spores as derived by qPCR, and rarefaction curves were again obtained by interpolating the diversity values with the number of spores using "iNEXT". Here, three precipitation classes were considered to be able to construct rarefaction curves (Low= P < 5 mm. (n=3), Moderate= P > 10 mm. (n=4), Severe= P > 100 mm. (n=1)). However, significance of precipitation effects on richness was tested, using precipitation as a continuous variable in LME models following the previously described schemes. Finally, to study the number of specific OTUs associated with each week, we used indicator species analyses (De Caceres and Legendre, 2009), using weeks' identity as explanatory factor (n=8). Here, values $P<0.05$ were considered significant.

3. Results

3.1 Fungal spore community composition and structure

We identified 521 OTUs belonging to 12 functional guilds. Based on relative abundances, OTUs belonging to undefined saprotrophs were the most abundant (16%), followed by plant pathogens (15%), soil saprotrophs (5%) and mycorrhizal species (4%). Based on OTU richness, the most abundant guilds were; undefined saprotrophs (209 OTUs), mycorrhizal (109 OTUs), plant pathogens (75 OTUs) and wood saprotrophs (41 OTUs; Table 1).

3.2 Spore community compositional changes across weeks and plots (succession)

The highest compositional changes in deposited spores occurred across weeks (weeks within one autumn season, Table S1), thus there was a strong spore succession across the 8 sampled weeks. Here, spore succession refers to a progression of spore community composition across the 8 autumn weeks for one year. While the temporal changes were consistently strong across all functional guilds with the exception of lichenized fungi, only ectomycorrhizal, wood saprotrophs and other saprotrophs showed also significant changes across plots. Nevertheless, temporal variation was twice as big as spatial variation (Table S1). Similarly, spore composition showed spatial autocorrelation, since shared variance of geographical distance and plot identity was about 8.6% (Fig. S3). Mycorrhizal fungi showed the highest spatial autocorrelation in comparison with pathogens, wood saprotrophs and other saprotrophs (group mostly represented by dung saprotrophs), since shared variance of plot identity and geographical proximity was about 3.2% of the 4.4% total variation explained by plot identiy (Fig. S3). Soil saprotrophs, wood saprotrophs and other saprotrophs were the guilds with greater relative abundance fluctuations across weeks, while wood saprotrophs displayed large fluctuations across space (Table 1, Table S2).

There was a succession in spore deposition from early October to early December (light blue circles vs. dark blue circles in Fig. 1, S4). The successional trend was especially evident for mycorrhizal fungi, saprotrophs and soil saprotrophs, and almost non-existent for lichenized species (Fig. 1). However, the successional changes of fruit body community were stronger (P<0.001, R^2= 72%, Fig. S5) than those of spores (P<0.001, R^2=18%; Fig. 1).

Trends in relative abundance of spores from early to late autumn were different across guilds (Fig. S6). Spores of mycorrhizal fungi and soil saprotrophs followed a U-shape pattern, whereas wood saprotrophs increased linearly and fungal parasites decreased with time (Fig. S6). In addition, there were also different species-specific fluctuations across weeks within the same guilds. For example, many saprotroph and ectomycorrhizal species followed a unimodal trend and were more abundant during the beginning of the sampling period (e.g. *Macrolepiota procera*, *Lactarius vinosus*, most of the *Suillus* sp.; Fig. 2) or at the end of the sampling period (e.g. *Tricholoma terreum*, *Lycoperdon perlatum*, *Inocybe* spp.; Fig. 2).

3.3 Rainfall and mushroom emergence effects on spore composition, diversity and abundance

Rainfall significantly affected the deposited spore composition (F=3.11, *P*=0.006, R^2= 4.7%). However, a detailed inspection of rainfall effects over deposited spore composition showed a significant negative correlation between rainfall and weekly compositional multivariate variance (i.e. beta diversity decreasing with precipitation (r= -44%): Fig. S7). Rainfall reduced the spatial heterogeneity of the deposited spores, making them more homogeneous during the rainy weeks (7 ± 4% of multivariate variance) than in non rainy weeks (low or no precipitation, < 5 mm, = 46 ± 7% and moderate precipitation, > 5 mm. = 26 ± 7%). Remarkably, the reduction of compositional multivariate variance in rainy weeks was identical in all sampled plots. In the same way, deposited spore diversity was also positively influenced by rainfall (analysed as continuous variable) and mushroom emergence (Table S3; Fig. S8). This positive effect of rainfall over spore diversity was still evident after correcting the relative proportions of each OTU by their total number of spores recorded in each trap (Fig. S8). Diversity was greatest during the 1st, 6th and 7th sampling week, coinciding with the three peaks in precipitation recorded. During the 7th sampling week, and overlapping with the

extreme rainfall event, spore diversity was especially higher than the other weeks (Fig. S8, S9). Species indicator analysis showed a greater number of OTUs associated with heavy rainy weeks (e.g. Week 1: 157 OTUs, Week 7: 252 OTUs) than weeks with lower or no rainfall (e.g. Week 5: 11 OTUs, Week 8: 30 OTUs).

Abundance of deposited spores quantified by qPCR was also positively associated with rainfall ($F_{[1,48]}$=8.10, *P*=0.006; Fig. 3A). This effect was again especially evident during the 7th week, when there was a strong peak in spore abundance following a large rainfall event (>150 mm/24 h). At functional level, rainfall associated positively with the relative abundance of soil saprotrophs ($F_{[1,48]}$=40.4, *P*=0.001) and undefined saprotrophs ($F_{[1,48]}$=5.2, *P*=0.028), whereas rainfall was negatively associated with fungal pathogens ($F_{[1,48]}$=12.4, *P*=0.001; Fig. 4A). Soil saprotroph group was mainly composed of OTUs forming puffballs. When studying the correlation of these and other species forming puffballs from other guilds, a great majority of them showed a high and significant positive correlation with rainfall (average across species of R^2=0.75, Fig. 3B, Fig. 4B).

4. Discussion

Our results using spore traps in a local-scale set up showed that deposited fungal spores were succesionally changing during autumn, following the fungal fruit body season. Rainfall positively influenced fungal spore deposition (whole spores), especially from soil saprotrophs and other saprotrophs. This positive correlation was especially high for puffballs. Spatial homogenization of deposited spore composition was related to rainfall, with higher spatial homogenization during wet weeks. Our findings point out the importance of both fruit body emergence and rainfall events in determining temporal spore deposition in Mediterranean forests, where both mushroom emergence and rainfall are irregular and often scarce events. It seems that predicted irregularity of both rainfall events and fruit body emergence in

Mediterranean regions (Alday et al., 2017) may cause potential alterations in fungal dispersal and deposition, potentially affecting fungal community renewal and colonization of Mediterranean forest ecosystems.

We observed variation in spore deposition amongst time and space, although temporal changes were more important than spatial changes at local scale. Changes in airborne spore composition at temporal scales are well-known (Kivlin et al., 2014; Nicolaisen et al., 2017; Abrego et al., 2018), and recent studies showed low spatial changes of airborne spores at local scale (Abrego et al., 2018). In our study, we showed that variation across time and space is highly affected by rainfall, which should be considered in future aerospore diversity studies. In addition, spore composition and diversity was also positively related to mushroom emergence (Castaño et al., 2017). Recent studies have also shown positive relationships between the presence of the pathogen *Diplodia sapinea* and its spore abundances in spore traps (Brodde et al., 2019), suggesting that spore traps may be useful to study the presence and the phenology of fungal species.

Significant positive rainfall relationships with specific species of fungal spores were previously reported (Oliveira et al., 2009; Peay and Bruns, 2014). In contrast, negative correlation between rainfall and airborne spores has been reported (Pakpour et al., 2015), suggesting that rainfall washed-out airborne spores, increasing deposited spores. There was a lack of knowledge about the effects of rainfall events on deposited spore diversity or the differential effects across functional guilds. Our local-scale study supports a positive effect of rainfall on spore deposition, with an increase in both diversity and number of spores trapped. In addition, the negative association between rainfall and beta diversity across weeks suggests that rainfall may be a driver of community homogenization in time and space. Rainfall often occurs irregularly amongst spatial units in Mediterranean areas, thus perhaps homogenization

is not as strong as in other environments where rainfall is more frequent and affects larger areas. Thus, the observed positive effect of rainfall on deposited spores could be especially relevant in Mediterranean forests, with potential ecological implications with respect to spore establishment.

Lack of strong dispersive winds may prevent fungal spores from reaching the turbulent boundary layer (Lacey and West, 2006). However, rainfall events are often accompanied by strong winds that may promote both spore mixing and deposition, thus, favouring the homogenization of airborne spores. In our study, we found a higher number of species during high rainfall events than during low rainfall events in autumn. We could speculate that in the heaviest rain events not only these spores lifted from the ground but they also arrived from distant sources or were present in higher atmospheric layers, such as the turbulent layer of the atmosphere (Lacey and West, 2006; Nicolaisen et al., 2017). However, at community level, successional changes in spore composition were much greater (R^2=28%) than compositional changes caused by rainfall (R^2= 4.7%), suggesting a small contribution of spores from the turbulent layer. Thus, it is possible that most of the spores gathered by rain originate from nearby sources, and just a small fraction from distant sources. Future studies employing larger scale sampling design schemes could help provide better understanding of the geographical origin of these spores.

New OTUs were exclusively found during specific rainfall events. This was most evident during the 7th week of sampling, as was shown by the high number of indicator species. The rain event of that week, was not only strong but also carried some Saharian dust, with estimated peaks of wet deposition around 50-400 mg of dust m^{-2} in only 3 h during the precipitation event (Barcelona Dust Forecast Center), which may potentially be the source of the new species. Similar observations were made by Reche et al., (2018), who showed that

deposition rates of bacteria were significantly higher during rain events and Saharian dust intrusions, with bacterial deposition rates ranging from 0.3×10^7 to $>8\times10^7$ m^{-2} per day. Thus, as already demonstrated with bacteria, it is possible that some spores coming from far distances were deposited in our plots, but in our study it is not possible to know which spores nor their abundance.

A high positive correlation between rainfall and deposited spores was found for soil saprotrophs and undefined saprotrophs. However, the highest correlation was found for puffball species (e.g. *Lycoperdon* spp. and *Bovista* spp.), included within the soil saprotrophs, which are assumed to be very efficiently transported by wind (Hitchcock et al., 2011). Interestingly, no association between fruit body production and spores was found in previous studies for these species (Castaño et al., 2017). These results suggest that, for this group of species, rainfall may be a driver for both spore ejection and deposition, since no spores were found outside rain events even though fruit bodies were present. Thus, despite this mechanism not being tested here, we hypothesize that raindrops during rain may hit the puffball and favor ejection. These results can be explained by the ecology of spore dispersal of each species. For example, species belonging to *Lycoperdon* are known to release spores when a raindrop hits the endoperidium, whereas some other species rely more on wind or animal trampling (Webster and Weber, 2007). We could hypothesize that climate can act as a selection driver of spores dispersal, and may explain the changes in spore composition across climate types.

This study provides new knowledge including understanding of how spatio-temporal changes in deposited spores and how rainfall affect fungal spore deposition. For example, successional changes in the fungal fruit body community partially paralleled successional changes in the deposited spores (Fig. 5A), which means that most of the spore deposition rely on mushroom emergence and gravity at short-time scales. However, previous studies have shown that a

small fraction of ejected spores potentially remains airborne or reaches dispersive winds (Dressaire et al., 2016), forming a spore bank in the atmospheric layer (seed-bank model theory; Reche et al., 2018). Among environmental factors, rainfall may contribute to increase both, (i) the amount and diversity of deposited spores, and (ii) the spore composition homogeneity amongst plots compared with drier weeks (Fig. 5B). Thus, it seems that in our local-scale study the rainfall events may have potentially washed-out the circulating atmospheric spores. In addition, some rainfall events may be a source of new spores coming from geographically distant sources (Fig. 5C). For some species, such as puffballs, rainfall may also promote spore ejection (Fig. 5D). These results provide a step further in predicting fungal assemblages in forest ecosystems. However, studies considering larger spatial and temporal scales could provide a better understanding of the relative importance of these processes.

Conflict of interests

The authors declare no conflict of interests associated with this publication.

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Catalunya, and by the European project StarTree (No. 311919). J.G.A. was supported by Juan de la Cierva (Grant number IJCI-2014-21393) and Ramon y Cajal fellowships (RYC-2016-20528), while JO was supported by Ramon y Cajal fellowship (RYC-2015-17459). José Antonio Bonet benefited from a Serra-Húnter Fellowship provided by the Generalitat of Catalunya. Carles Castaño received the support of the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya through the program of Doctorats Industrials, and benefited from a STSM grant from COST Action FP1203.

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Figures

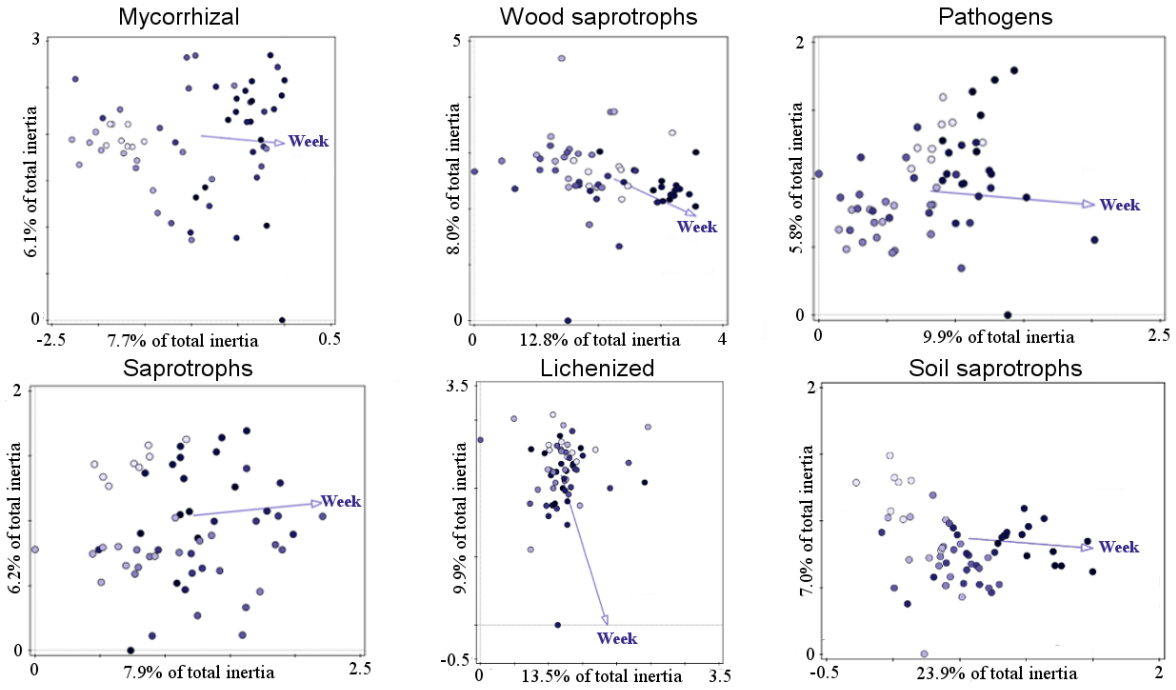

Fig. 1. DCA analysis of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons, showing variation in deposited spore composition across weeks. Here, 'week' was defined as supplementary variable. Shift from light blue to dark blue in colour represents a gradient from the beginning of sampling season to final of sampling season.

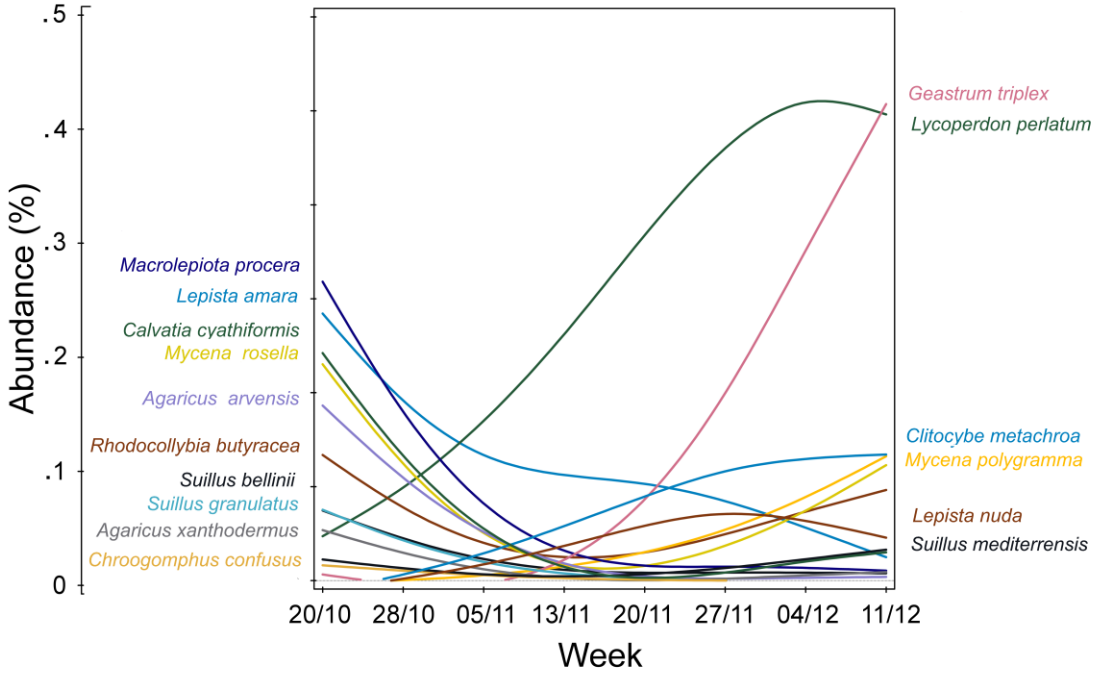

Fig. 2. Species-response curve using GAM models of the most abundant mycorrhizal and saprotrophic species. Weeks are shown on the x-axis, starting from the 1st sampling week to the last week.

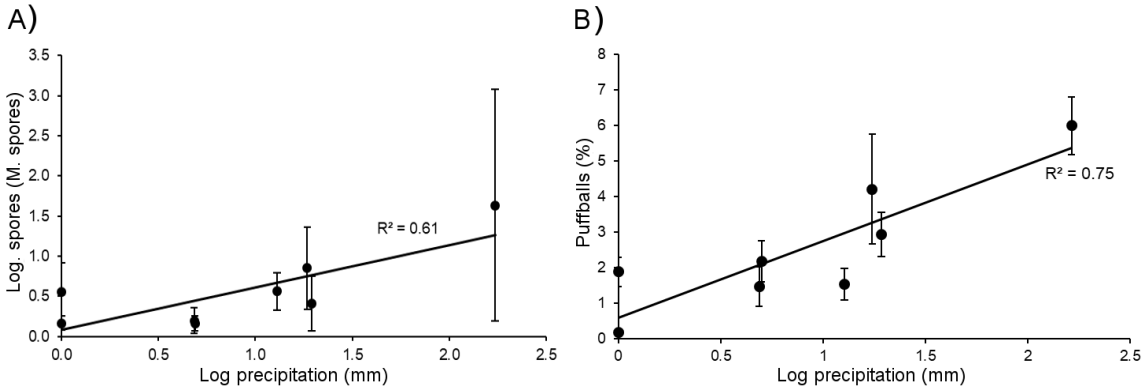

Fig. 3. Correlation between weekly averaged precipitation and (A) the total number of spores recorded at the spore traps, and (B) the relative abundance of puffballs. Error bars indicate standard error.

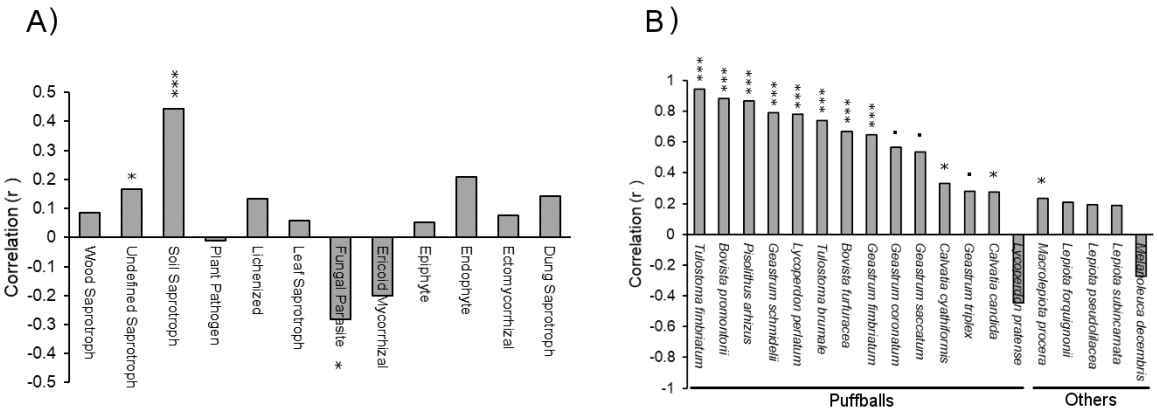

Fig. 4. (A) Pearson correlation between weekly precipitation and the relative abundance of each guild and, (B) correlation between weekly precipitation and relative abundance of each species belonging to soil saprotrophs, including puffball species and non-puffball species (others). In (b), puffballs belonging to other functional guilds are also included (i.e. *Pisolithus arhizus* and *Lycoperdon* spp.). Significance values: "*" $P<0.05$, "**" $P<0.01$, "***" $P<0.001$.

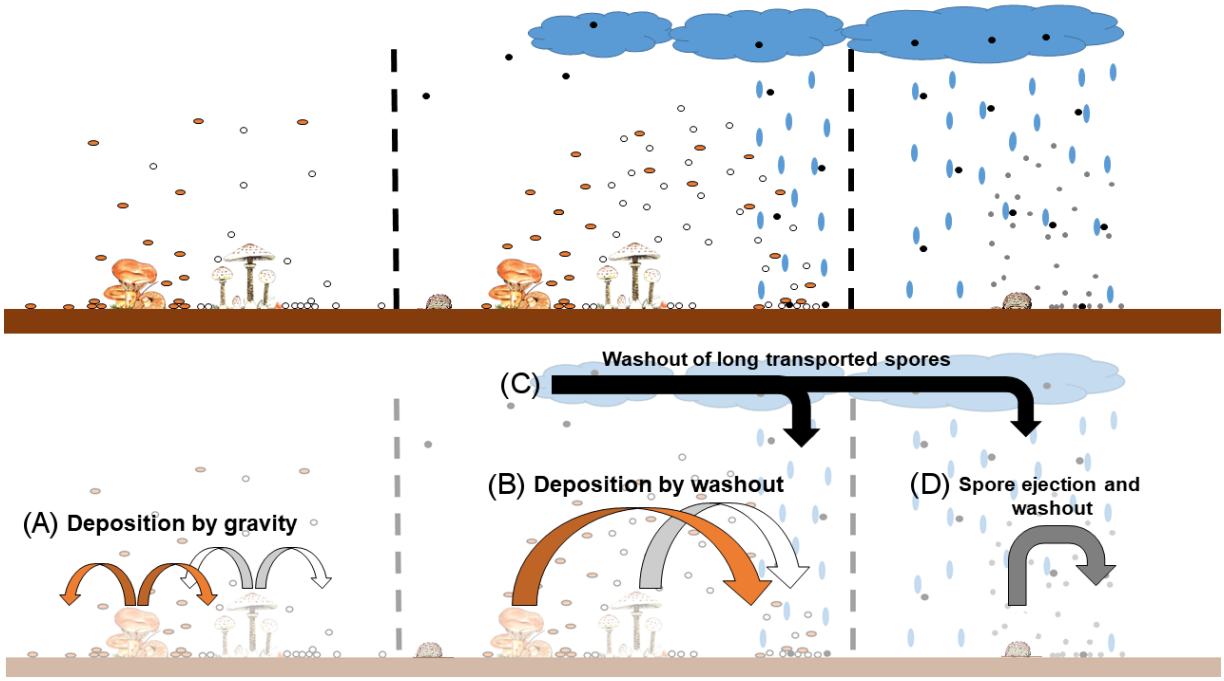

Fig. 5. Summary of the drivers influencing spore deposition observed in this study. Billions of spores are produced by many species and a very important fraction of them may fall close to the spore source, despite a small fraction of them still reaching dispersive winds (A). Here, we suggest that rainfall washed-out the atmospheric spores, concurrent with the greater spore diversity and abundance during rainy days (B). Winds and clouds may transport particles from sites located further (e.g. the week from 27th November to 4th December) that may fall during the rainfall event (C). Finally, rainfall may not only promote spore deposition but also cause spore ejection (e.g. puffballs; D).

Tables

Table 1. Functional guilds detected at the spore traps, and the number of OTUs and % of reads that represent each functional guild. The most abundant species and the most diverse genera within the guild are also shown. Guilds were identified by FUNGuild and 45.88% of the reads could not be ecologically identified and they were unassigned.

Guild	Richness	% reads	Most abundant species	Most diverse genera
Undefined saprotroph	209	16.27	*Phaeococcomyces catenatus*	*Agaricus, Mortierella*
Ectomycorrhizal	109	3.97	*Suillus bellinii*	*Cortinarius, Tomentella, Russula*
Plant pathogen	75	14.92	*Mycosphaerella tassiana*	*Melampsora, Puccinia*
Wood saprotroph	41	1.87	*Crepidotus cesatii*	*Postia, Trechispora*
Soil saprotroph	21	5.14	*Bovista promontorii*	*Lepiota*
Lichenized	21	0.72	*Physcia stellaris*	*Hyperphyscia, Lecanora, Ramalina*
Fungal parasite	19	3.07	*Sporobolomyces oryzicola*	*Sporobolomyces*
Leaf saprotroph	13	7.98	*Phallus impudicus*	*Mycena*
Ericoid mycorrhizal	4	0.07	*Oidiodendron sp.*	*Oidiodendron*
Endophyte	4	0.05	*Cadophora epimyces*	*Cadophora*
Animal pathogen	3	0.02	*Beauviera bassiana*	*Beauviera*
Epiphyte	2	0.03	*Bullera sp.*	*Bullera*
TOTAL	521	54.120		

Supplementary material

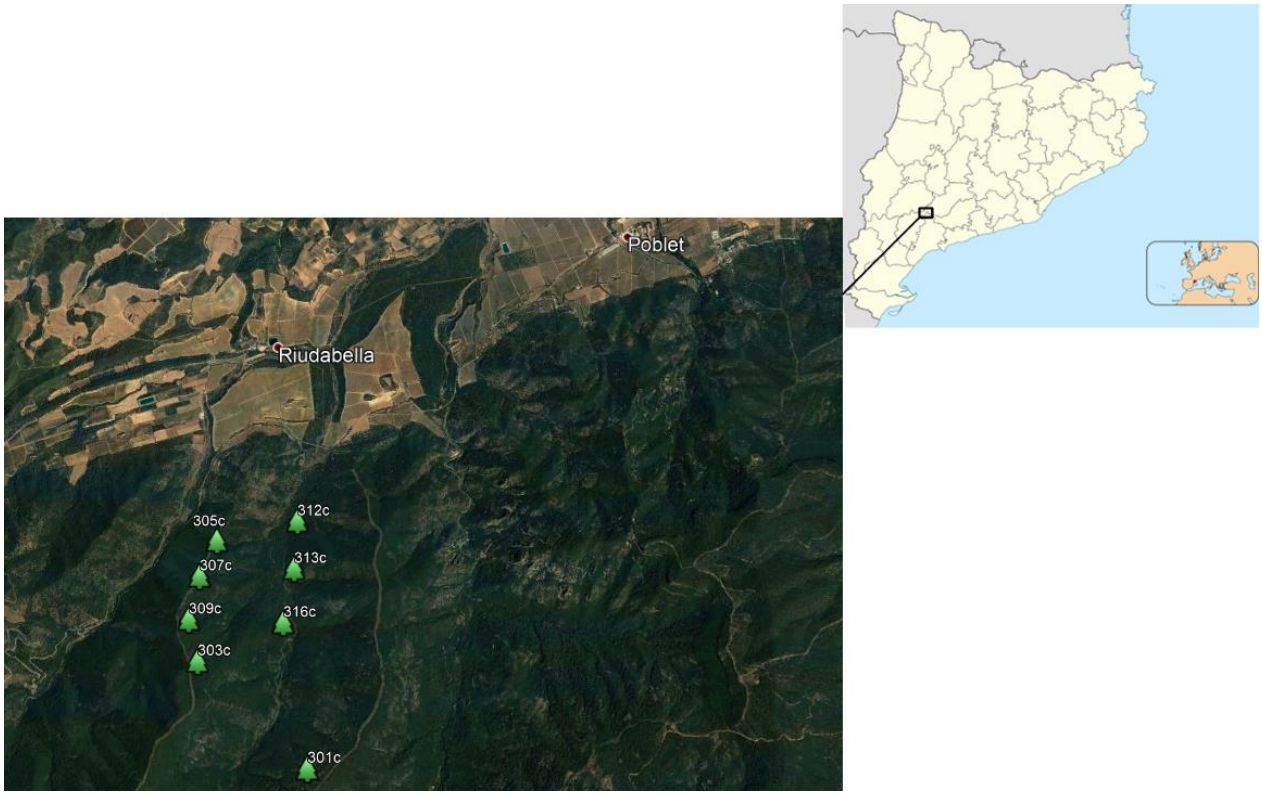

Fig. S1. Geographical localization of the study plots.

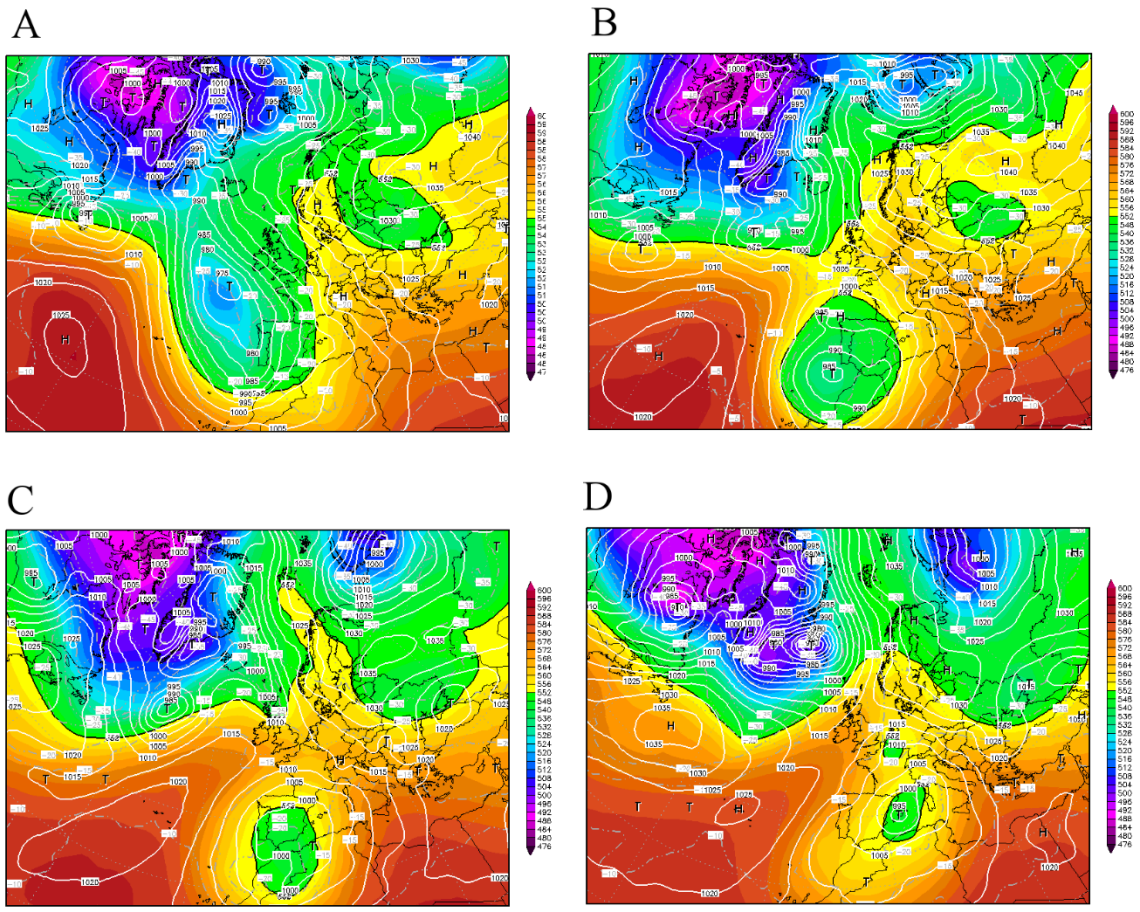

Fig. S2. Synoptic geopotential maps at 500 hPa during the days 28 November (A), 29th November (B), 30th November (C) and 1st December (D) at European scale. Maps show a strong cut-off low in the Iberian Peninsula, as a result of the isolation of cold air temperatures at high altitudes (lower than -25ºC at 5,000 m.) from the *polar jet stream* and subsequent isolation by an undulated belt with high pressures in central Europe. The influence of this cut-off low, together with humid winds with sea influence (note the SE winds in B and C) resulted in rainfall events >160 mm in less than 48 h in our study plots. Sequential geopotential maps were obtained using the "reanalysis" option from the Global Forecast System (GFS) at www.wetterzentrale.de. Colours indicate a gradient in geopotential values. "H" indicate high air pressures and "T" low air pressures. In "H", air direction at the isolines follow clock-wise direction whereas in "T" air direction follows counter clock-wise directions. Here, especially in B and C, maps indicate strong S-SE air directions that carried significant amounts of dust coming from North Africa.

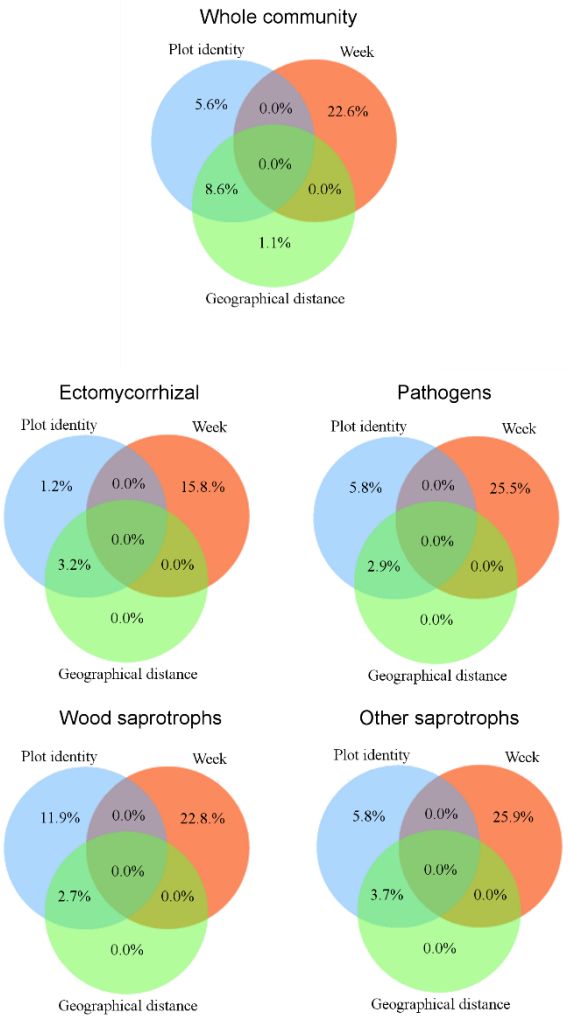

Fig. S3. Variation partitioning analysis considering the spores from the whole community, ectomycorrhizal species, pathogens, wood saprotrophs and other saprotrophs. In these analyses, plot identity, week and geographical distance factors are tested.

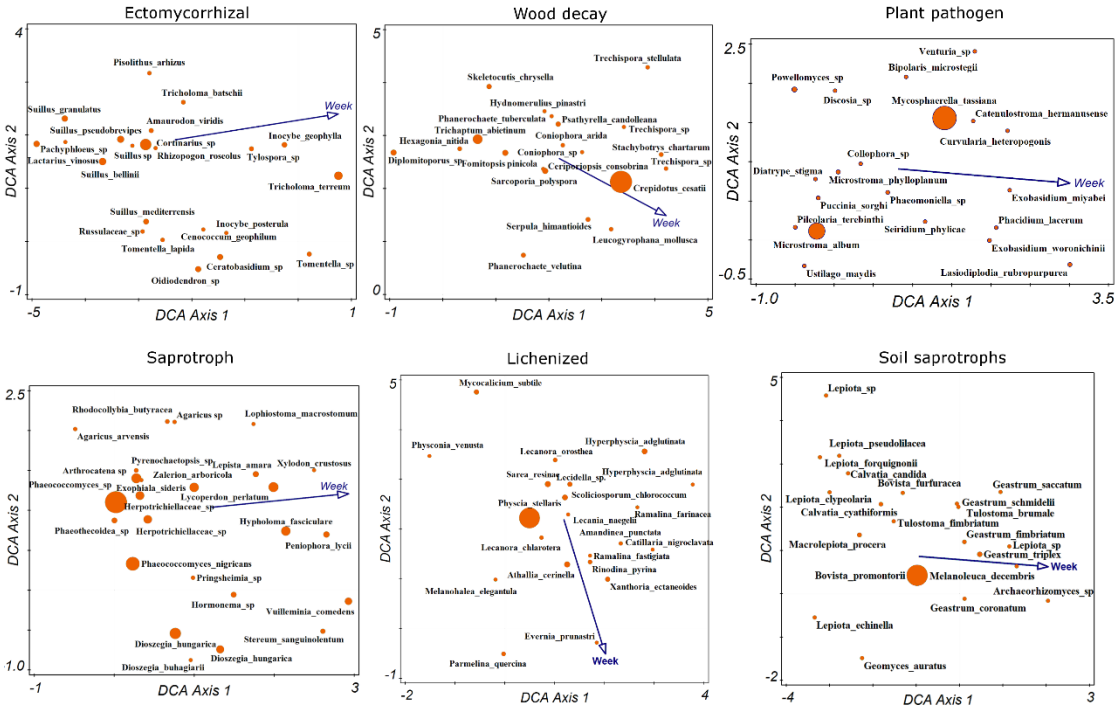

Fig. S4. Species biplots DCA analyses of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons. The figures illustrate variation in taxa occurrence of spores across weeks. Here, ‘week’ was defined as supplementary variable. Symbol size is proportional to the relative abundance of the given taxa.

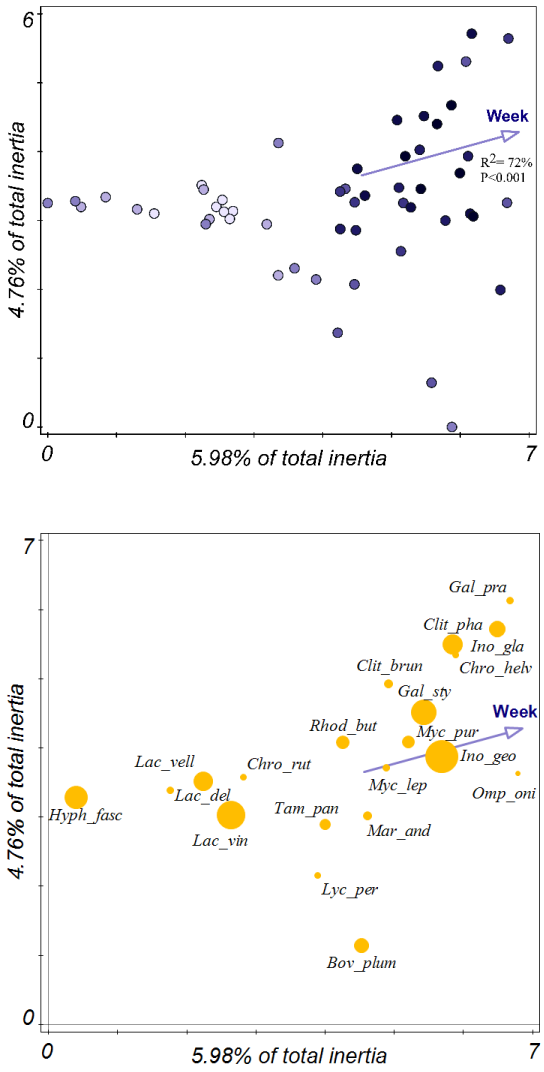

Fig S5. DCA analysis of the fruiting body community composition in a Mediterranean pine forest, with (a) the sample plot and (b) the species plot showing the 20 most abundant species. The figures illustrate changes in fruiting body community composition across weeks. Here, ‘week’ was defined as supplementary variable. Shift from light blue to dark blue in colour represents a gradient from the beginning of sampling season to final of sampling season. Symbol size in the species plot is proportional to the relative abundance of the given taxa.

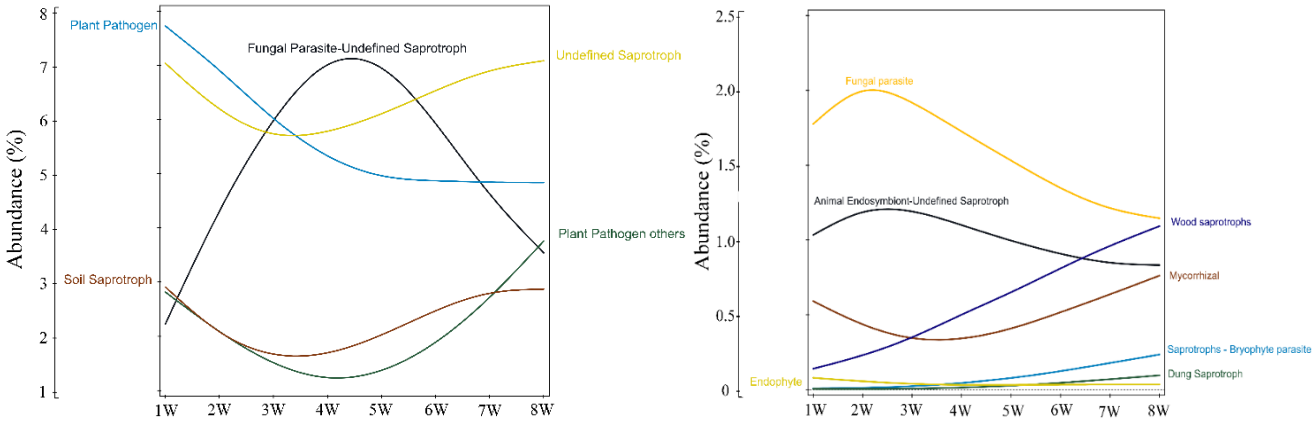

Fig. S6. Temporal fluctuation of the functional guilds that showed significant changes across weeks and visualized by GAM models.

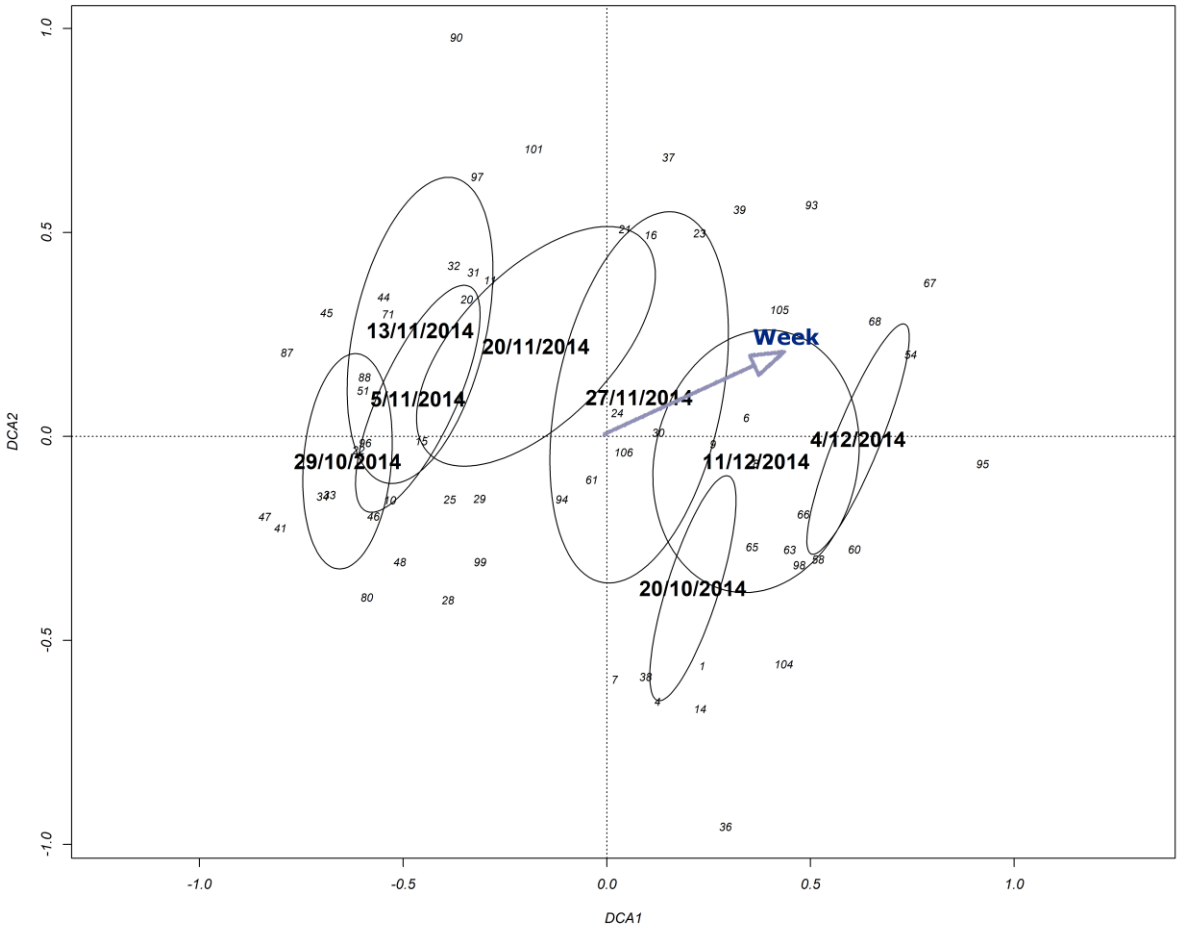

Fig. S7. DCA analysis of the deposited fungal spore composition in a Mediterranean pine forest, as analysed by sequencing of ITS2 amplicons, considering all the fungal guilds. Ellipses show data heterogeneity and data dispersion across weeks. Narrow ellipses indicate low heterogeneity, meaning that compositional changes across plots within each week are low.

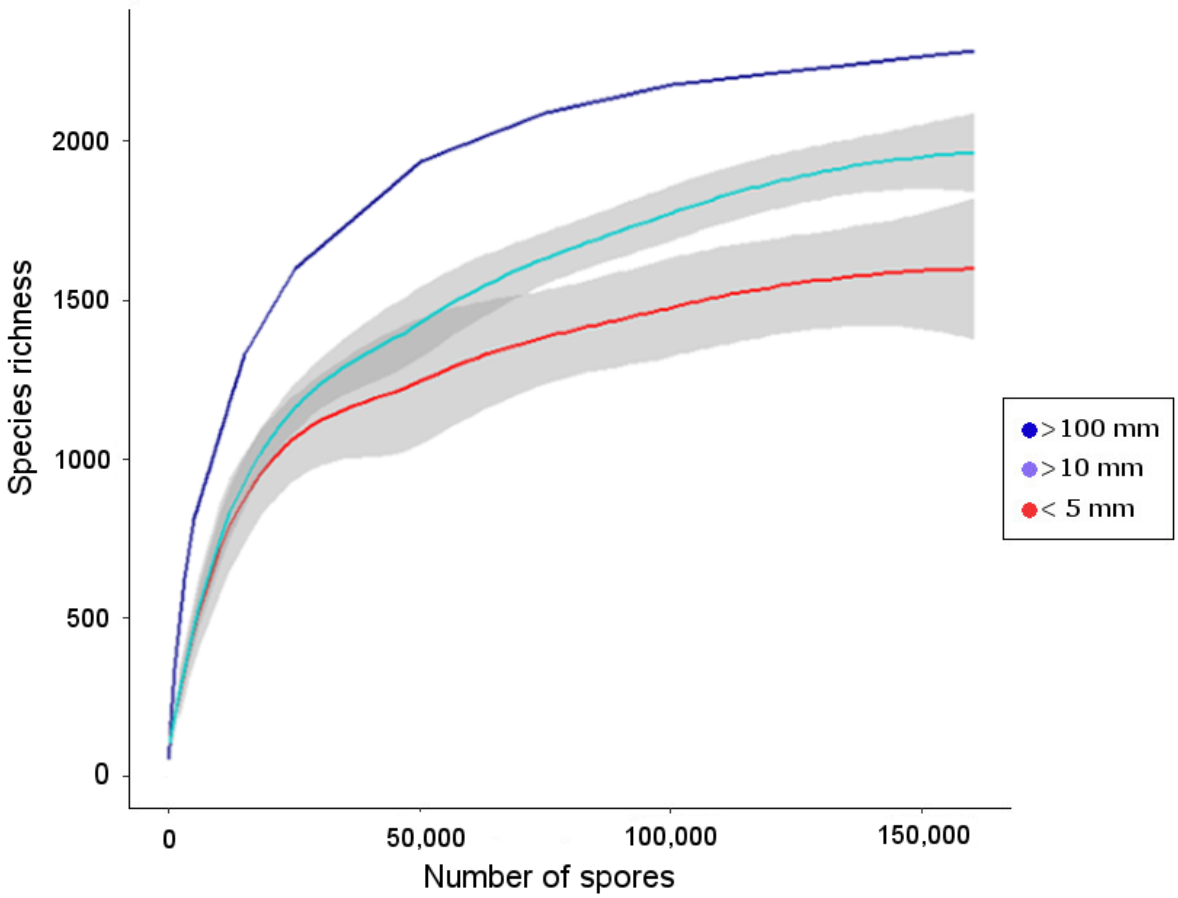

Fig. S8. Rarefaction curves based on the ITS relative proportions of each species as evaluated by Illumina MiSeq, and corrected by the total number of spores as evaluated by qPCR. Here, we considered three precipitation classes: (Low= P < 5 mm., Moderate= P > 10 mm., Severe= P > 100 mm.). Here three precipitation classes were considered, but statistical significance of precipitation effects on richness was tested over precipitation values taken as a continuous variable.

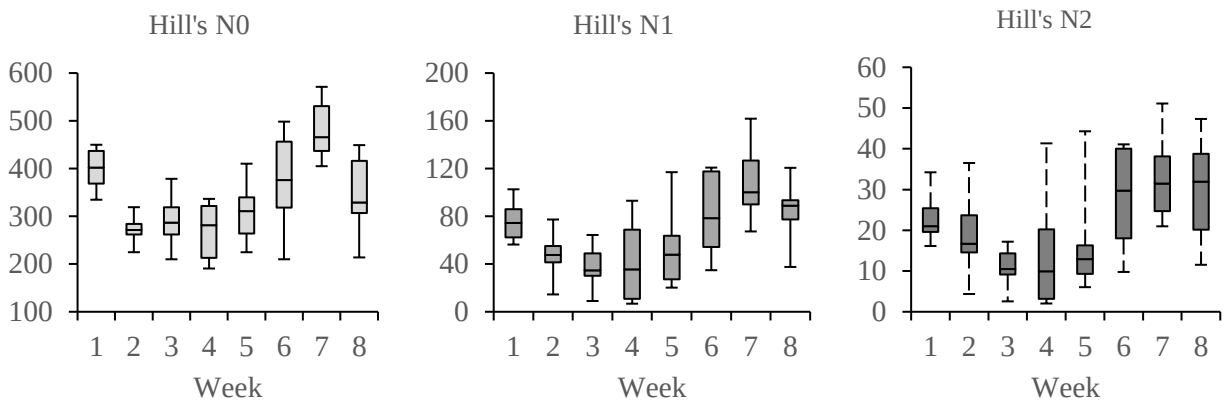

Fig. S9. Hill's diversity numbers of the whole fungal spore composition across the 8 sampling weeks.

Table S1. Variation partitioning analyses considering the temporal (a) vs spatial (b) variation in total deposited spore composition and splitted by functional guilds.

	Temporal variation (a)	Spatial variation (b)	
	Adj R^2	Adj R^2	Residuals
Whole composition	**0.23(*P*<0.001)**	**0.14(*P*<0.001)**	0.63
Functional guilds			
Ectomycorrhizal	**0.16(*P*<0.001)**	**0.04(*P*=0.045)**	0.80
Wood saprotrophs	**0.23(*P*<0.001)**	**0.15(*P*<0.001)**	0.62
Pathogens	**0.25(*P*<0.001)**	0.09(*P*=0.053)	0.66
Other saprotrophs	**0.26(*P*<0.001)**	**0.10(*P*<0.001)**	0.66
Lichenized	**0.06(*P*<0.009)**	0.04(*P*=0.063)	0.90
Soil saprotrophs	**0.49(*P*<0.001)**	0.07(*P*=0.487)	0.51

Table S2. Temporal changes in relative abundance of each functional guild, analysed by GAMM models. Here, relative abundance of each functional guild was considered the sum of each OTUs belonging to the same guild.

	Temporal changes		
Guilds	**F**	***P*-value**	**Adj. R^2**
Animal pathogen	1.98	**0.011**	**8.6**
Moulds	0.05	0.946	1.7
Dung saprotrophs	19.57	**<0.001**	**43.1**
Mycorrhizal	6.25	**0.001**	**21.8**
Endophyte	7.36	**<0.001**	**23.3**
Epiphyte	9.91	**0.020**	**10.4**
Fungal parasite	0.17	0.678	1.4
Lichenized	0.61	0.436	0.1
Plant pathogen	8.28	**0.006**	**8.1**
Soil saprotrophs	12.67	**<0.001**	**46.7**
Undefined saprotrophs	6.2	**<0.001**	**21.6**
Wood saprotrophs	36.06	**<0.001**	**34.4**

Table S3. Effect of fruiting body emergence (Richness), rainfall and its interaction on Hill's numbers derived from the spore community, as evaluated by LME, and considering subsampled composition to the sample with the lowest number of reads.

		Richness (N0)		Shannon (N1)		Simpson (N2)	
Effects	**dF**	**F-value**	***P*-value**	**F-value**	***P*-value**	**F-value**	***P*-value**
Intercept	1	675.20	**<0.001**	97.4	**<0.001**	72.1	**<0.001**
Fruiting bodies (FB)	1	9.56	**0.003**	8.95	**0.043**	4.50	**0.039**
Rainfall	1	10.68	**0.001**	4.94	**0.031**	1.25	0.268
FB × Rainfall	1	11.49	0.309	0.23	0.635	0.10	0.752